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Dansylcadaverine and Cytochalasin D Enhance Rotavirus Infection of Murine L Cells

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Although murine L cells bind and internalize rotavirus as well as permissive cell lines, L cells are essentially nonpermissive for rotaviruses. In nonpermissive cell lines such as L cells, internalized rotavirus fails to uncoat and remains as infectious, double-shelled particles. This block in the infectious cycle can be overcome by direct lipofection of viral particles into the L cell cytoplasm. We hypothesized that the internalized rotavirus particles within L cells are sequestered in the endocytic pathway and are unable to initiate infection. L cells were pretreated with a variety of inhibitors of endocytosis prior to infection with rhesus rotavirus. While agents which inhibit acidification of endosomes had no effect on rotavirus infection, two potential direct inhibitors of vesicular transport, dansylcadaverine and cytochalasin D, enhanced rotavirus infection of L cells 5- to 10-fold. All of the drugs, including both inhibitors of endocytosis and lysosomotrophic agents, significantly reduced infection of L cells by serotype 1 reovirus which is known to infect L cells by the endocytic pathway. Time course studies demonstrated that the drugs were effective in promoting rotavirus infection of L cells in only the early phases of infection. Pretreatment of L cells with dansylcadaverine significantly decreased the number of intact, double-shelled rotavirus particles sequestered within the cells. Inhibition of endocytosis may increase the efficiency of infection of L cells by rotavirus by allowing an increased proportion of attached rotavirus virions to enter cells by a productive route which is probably direct membrane penetration.

INTRODUCTION

Rotaviruses are ubiquitous enteric pathogens of mammals and birds. Rotaviruses cause diarrhea in their hosts by selectively infecting intestinal epithelial cells, specifically the highly differentiated cells on the upper portion of the small intestinal villi. Why undifferentiated small intestinal cells and other epithelia such as the respiratory tract are not infected is not known. One level at which cell tropism for a particular virus may be expressed is at the inception of infection during viral attachment and entry into a potential target cell. Although much has been learned about the entry of enveloped viruses in recent years (reviewed in Marsh and Helenius, 1989), much less is known about the mechanisms by which icosahedral viruses reach their site of intracellular replication. Ultrastructural studies of rotavirus entry into cells have shown evidence both for endocytosis (Ludert *et al.*, 1987; Quan and Doane, 1983) and for direct membrane penetration (Suzuki *et al.*, 1985, 1986). Other studies have shown a lack of significant inhibitory effect of lysosomotrophic agents on rotavirus infection in permissive MA 104 cells (Fukuhara *et al.*, 1987; Kaljot *et al.*, 1988; Ludert *et al.*, 1987).

In previous studies, we have shown that rotavirus binds to both permissive and nonpermissive tissue culture cells in similar amounts (Bass *et al.*, 1992). Furthermore, internalization of the bound virus, as measured by escape from external proteolytic digestion, was similar in both types of cell lines. Examination of the internalized virus revealed that in nonpermissive cells (such as murine L cells) much of the internalized virus remained fully infectious while virus internalized by permissive cells such as MA 104 cells was not infectious. Thus rotavirus virions failed to uncoat in nonpermissive cells. However, introduction of intact rotavirus particles into nonpermissive cells via lipofection resulted in efficient viral replication. These results suggested that in nonpermissive cells, internalized rotavirus particles are sequestered in a noncytoplasmic compartment such as endosomes/lysosomes and are unable to reach their site of uncoating and replication in the cytosol. The present report describes experiments designed to further explore the block to rotavirus replication in L cells.

MATERIALS AND METHODS

Cells and viruses

Rhesus rotavirus (RRV) and the UK strain of rotavirus were grown in MA104 cells and purified by hydrofluorocarbon extraction and isopycnic centrifugation as previously described (Shaw *et al.*, 1986). Reovirus serotype 1 was obtained from Dr. Bernard Fields (Harvard Medical School) and grown in L 929 cells (L cells), obtained from ATCC. All cells were grown in Dulbecco modified minimal essential media (DMEM) supplemented with 10%

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fetal calf serum, L-glutamine, penicillin, and streptomycin in a 5% CO_2 incubator.

Infection of cells with RRV

Cells were grown to confluence in 96-well plates and washed twice with serum-free media prior to inoculation. Rotavirus was trypsin treated with 5 μg/ml trypsin (Type IX, Sigma) for 30 min at 37° prior to inoculation. Each well received enough virus in 50 μl of media to yield approximately 50 immunoperoxidase-positive cells under control conditions (no toxin added). For L cells this required approximately 100 times more virus than for permissive MA 104 cells. For experiments with toxins, cells were treated for 1 hr prior to inoculation with serial twofold dilutions of the toxins dissolved in serum-free DMEM. Starting concentrations were: Na azide, 100 m*M;* cytochalasin D, 20 μg/ml; colchicine, 1 m*M;* dansylcadaverine, 1 m*M;* NH_4Cl, 100 m*M;* methylamine, 50 m*M;* chloroquine, 10 m*M.* All toxins were from Sigma Chemical Co. (St. Louis, MO). In some experiments the toxins were removed after 3 hr and replaced with medium containing a potent neutralizing monoclonal antibody, 159, to neutralize any virus which had not entered the cells (Kaljot *et al.,* 1988). After 15 hr incubation at 37°, media was aspirated, the cells were fixed with ice-cold methanol, and infected cells were detected by immunoperoxidase staining as described (Kaljot *et al.,* 1988). Experiments with reovirus were carried out in an identical fashion except that the virus was not proteolytically activated. Toxicity to cells from the drug treatments was monitored by microscopy and trypan blue exclusion. Results were recorded only for drug concentrations with monolayers demonstrating greater than 90% viability. All experiments were performed at least three times, and representative results form single experiments are reported under Results.

Binding and internalization of RRV

Confluent monolayers in 24-well dishes were washed twice and chilled to 4°. Metabolically [^{35}S]methionine-labeled RRV (100,000 cpm/well, approximately 10^6 peroxidase focus units/well, 10 PFU/cell) was added and the monolayers were incubated at 4° with gentle rocking for 1 hr. For measurement of binding, the monolayers were washed three times with cold serum-free media, lysed with 2% SDS, and counted as previously described (Ruggeri and Greenberg, 1991). Under these conditions approximately 10% of the radiolabeled virus bound to the monolayer. Internalization was determined by binding the ^{35}S-labeled virus as above, followed by warming to 37° for 60 min. The cells were then treated with 500 μg/ml proteinase K for 30 min at 4°, washed twice with ice-cold media containing 2 m*M* PMSF and 10% FCS, lysed, and counted. Control experiments showed that 95% of the virus bound at 4° and not allowed to internalize was removed under these conditions.

Infectivity of internalized RRV

Confluent monolayers were washed with serum-free DMEM and infected with RRV (5 PFU/cell) at 4° for 1 hr in the absence or presence of 0.25 m*M* dansylcadaverine. The monolayers were washed again and warmed to 37° for 30 min and 1 hr. After washing again, neutralizing monoclonal antibody 159 ascites fluid was added to a 1:500 final dilution at 4° for 30 min. This treatment has been shown to neutralize all cell surface-bound RRV if the cells are not warmed to permit viral entry (Kaljot *et al.,* 1988; Ruggeri and Greenberg, 1991). The monolayers were washed three times more and the cell-associated infectious virus was recovered by freeze-thawing once and passing the lysate repeatedly through a 27-gauge needle. The resulting lysate was titrated on MA 104 cells by peroxidase focus counting. Control wells (total virus bound) were harvested after binding without monoclonal antibody treatment or warming.

Electron microscopy

L cells were grown on Costar Transwell filter inserts to confluence as previously described (Svensson *et al.,* 1991) and washed with serum-free medium. The cells were pretreated for 1 hr at 37° with 0.25 m*M* dansylcadaverine or mock-treated and were chilled to 4°. Purified RRV rotavirus was added at a multiplicity of infection of 200 and allowed to bind for 90 min at 4°. The medium was aspirated and replaced with 37° medium with or without dansylcadaverine, and internalization was allowed to proceed for 30 min at 37°. The cells were fixed in glutaraldehyde, processed, and examined by electron microscopy as described (Bass *et al.,* 1988).

RESULTS

Previous data from our laboratory suggested that rotavirus binds to and is internalized by nonpermissive murine L cell fibroblasts (Bass *et al.,* 1992). In these studies if rotavirus was introduced to these cells via lipofection, the cells were fully permissive for rotavirus replication. We hypothesized that the virus which was internalized in these nonpermissive cells was somehow trapped in an endocytic pathway which was not the productive route of infection. We tested this hypothesis by treating nonpermissive L cells with a variety of drugs which may disrupt the endocytic process to determine whether such treatment would render the cells more permissive.

Cytochalasin D and dansylcadaverine enhance rotavirus infection of L cells

When L cells were treated with various concentrations of the drugs 1 hr prior to and during RRV infection, infection of the cells as determined by immunoperoxidase staining increased 5- to 10-fold in cells exposed to dansylcadaverine and cytochalasin D compared to control

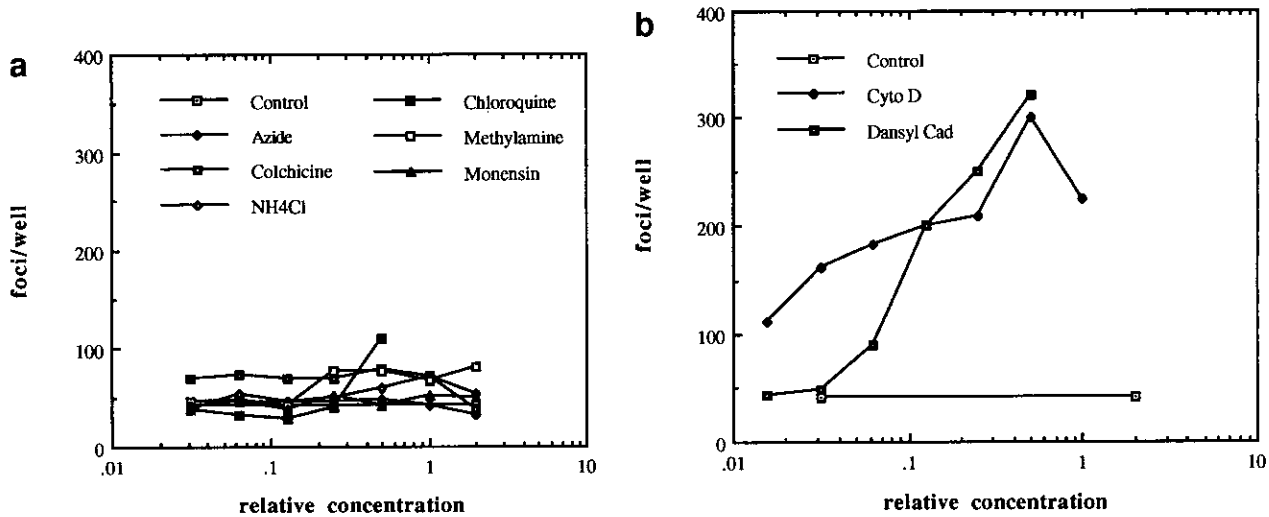

FIG. 1. (a) Effect of various lysosomotropic agents on RRV replication in L cell fibroblasts. Cells were pretreated with indicated dilutions of stock inhibitors for 1 hr prior to infection with RRV rotavirus. Starting concentrations were: Na Azide, 100 m*M*; cytochalasin D, 20 μg/ml; colchicine, 1 m*M*; dansylcadaverine, 1 m*M*; NH_4Cl, 100 m*M*; methylamine, 50 m*M*; chloroquine, 10 m*M*. Three hours after infection external virus was neutralized by addition of monoclonal antibody 159 for 1 hr. The cells were then washed with serum-free medium and infection was allowed to proceed overnight. The cells were then fixed with methanol and immunoperoxidase stained to enumerate infected cells. Data points represent means of three separate wells with the range of values less than 20% of the mean. (b) Effect of dansylcadaverine and cytochalasin D on RRV replication in L cell fibroblasts. Cells were pretreated with indicated dilutions of stock drugs (as in Fig. 1a) for 1 hr prior to infection with RRV rotavirus. Three hours after infection external virus was neutralized by addition of monoclonal antibody 159 for 1 hr. The cells were then washed with serum-free medium and infection was allowed to proceed overnight. The cells were then fixed with methanol and immunoperoxidase stained to enumerate infected cells. Data points represent means of three separate wells with the range of values less than 20% of the mean.

(untreated) cells (Fig. 1). The response appeared to be dose-dependent, especially for dansylcadaverine. None of the other drugs, including weak bases (methylamine, ammonium chloride, and chloroquine), microtubule inhibitors (colchicine and taxol), and an energy inhibitor (azide), exhibited a significant effect at concentrations tolerated by the cells. The results shown are for experiments in which exposure to the drugs was limited to the initial two or three hours of infection. Similar results were obtained for continuous treatment of the cells.

When the same panel of drugs was used to treat rotavirus permissive MA 104 cells, no significant change in viral infectivity was noted (Fig. 2). These results are consistent with prior studies (Fukuhara *et al.*, 1987; Kaljot *et al.*, 1988; Ludert *et al.*, 1987).

Serotype 1 reovirus infection of L cells is inhibited by a variety of drugs affecting endocytosis

In order to determine whether the effects of dansylcadaverine and cytochalasin D were specific for rotavirus infection or merely represented nonspecific toxic alteration of the L cells, identical experiments were carried out with serotype 1 reovirus. Reovirus, another icosahedral, encapsidated member of the Reoviridiae, has been previously shown to infect L cells via the endocytic pathway (Sturzenbecker *et al.*, 1987). The results of such an experiment are shown in Fig. 3. Treatment with all of the drugs except colchicine dramatically reduced infection of L cells by reovirus serotype 1. In parallel experiments, the same drugs inhibited reovirus infection of MA 104 cells (data not shown). Thus the same concentrations of dansylcadaverine and cytochalasin D in Fig. 3 which strongly inhibited the reovirus infection of L cells by an endocytic route strongly enhanced infection of L cells by a structurally similar virus, rotavirus (Fig. 1).

Dansylcadaverine exerts its effect on L cell rotavirus infection early in the replicative cycle

In order to determine at what point during viral replication dansylcadaverine enhances rotavirus infection of L cells, the drug was added to L cells at various times prior to and during infection. The results of such an experiment, shown in Fig. 4, demonstrated that dansylcadaverine significantly enhances rotavirus infection of L cells only if added during the first 30–45 min postinoculation.

Dansylcadaverine and cytochalasin D reduce the accumulation of intact RRV particles in L cells

We have previously demonstrated that large numbers of intact rotavirus particles accumulate in nonpermissive cells such as L cells when compared to permissive MA 104 cells (Bass *et al.*, 1992). We wished to determine whether treatment with dansylcadaverine might reduce this sequestration of double-shelled rotavirus particles which cannot initiate transcription. In Fig. 5 the results of such an experiment are depicted. L cells were treated with either 0.25 m*M* dansylcadaverine or 10 μg/ml cyto-

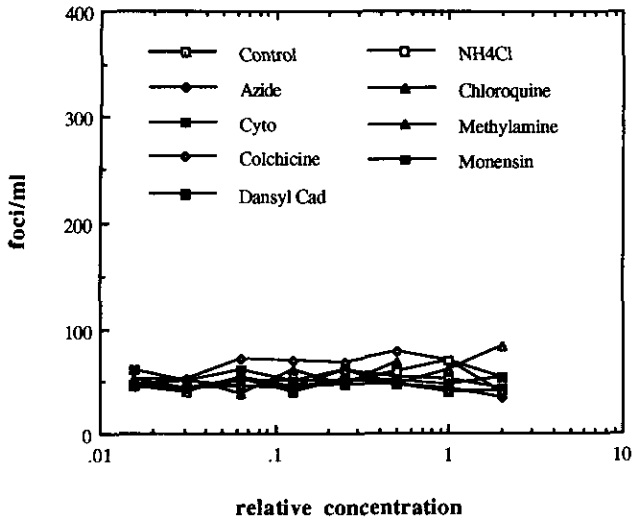

FIG. 2. Effect of various inhibitors of endocytosis on RRV replication in MA 104 cells. The experiment was performed as described in Fig. 1 and under Materials and Methods. Data points represent means of three separate wells with the range of values less than 20% of the mean.

chalasin D or mock-treated with media (control). At 30 and 60 min after warming the cells to 37°, the control cells accumulated significantly more internalized virus which scored as infectious when titered on MA 104 cells than did the treated cells (Fig. 5). Thus dansylcadaverine and cytochalasin D reduced the accumulation of fully coated transcriptionally inactive RRV particles within L cells. The other drugs had no significant effect on the accumulation of intact, infectious particles in L cells.

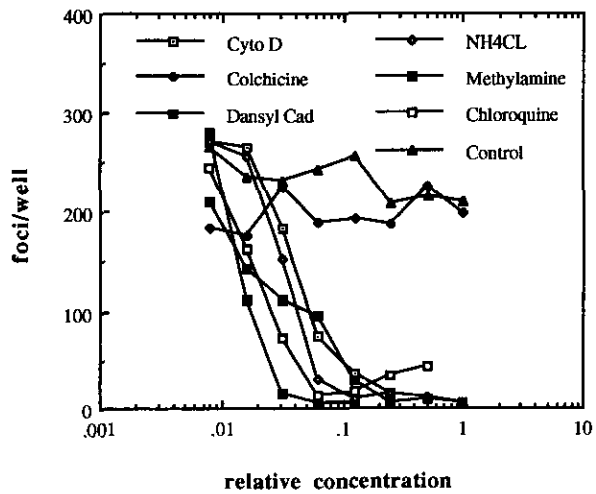

FIG. 3. Effect of various inhibitors of endocytosis on serotype 1 reovirus replication in L cell fibroblasts. Cells were pretreated with indicated dilutions of stock inhibitors for 1 hr prior to infection with RRV rotavirus. The drugs remained in the media throughout the infection. The cells were then fixed with methanol and immunoperoxidase stained to enumerate infected cells. Data points represent means of three separate wells with the range of values less than 20% of the mean.

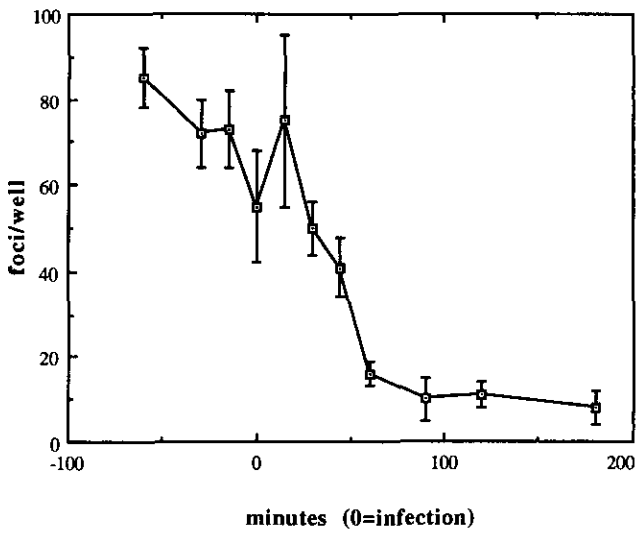

FIG. 4. Effect of adding dansylcadaverine to L cells infected with RRV rotavirus at various times during infection. Dansylcadaverine (0.25 m*M*) was added to L cell monolayers at various times prior to and during infection with RRV. Zero time is the time of infection. Infected cells were enumerated as described by immunoperoxidase staining. Control (not treated) wells in this experiment contained 13 infected cells/well. Error bars indicate SEM for three replicate wells for each time point.

Dansylcadaverine and cytochalasin had little effect on L cell binding and internalization of RRV

Because the enhanced permissivity of L cells treated with the drugs could be due to enhanced binding or internalization of the virus, we used purified ^{35}S-labeled

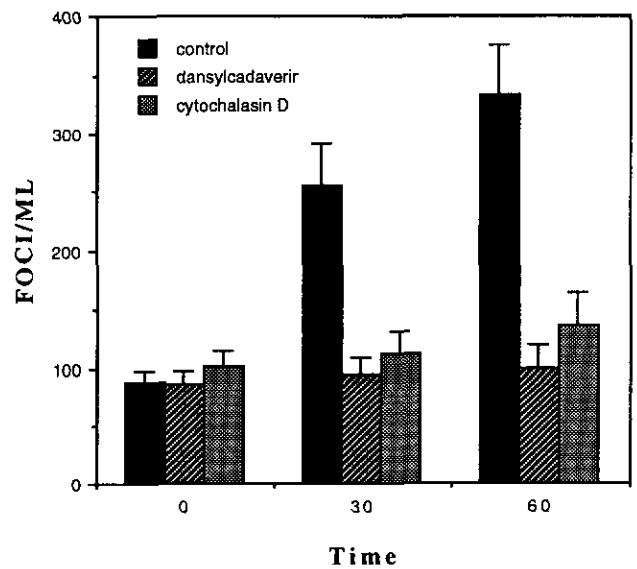

FIG. 5. Effect of dansylcadaverine on L cell internalization of infectious RRV rotavirus. L cell monolayers were pretreated with either dansylcadaverine (0.25 m*M*) or mock-treated prior to infection with RRV. At the indicated times the cells were chilled to 4°, treated with neutralizing monoclonal antibody 159, washed, lysed, and titered on MA 104 cells as described under Materials and Methods. Error bars indicate SEM for three replicate wells for each time point.

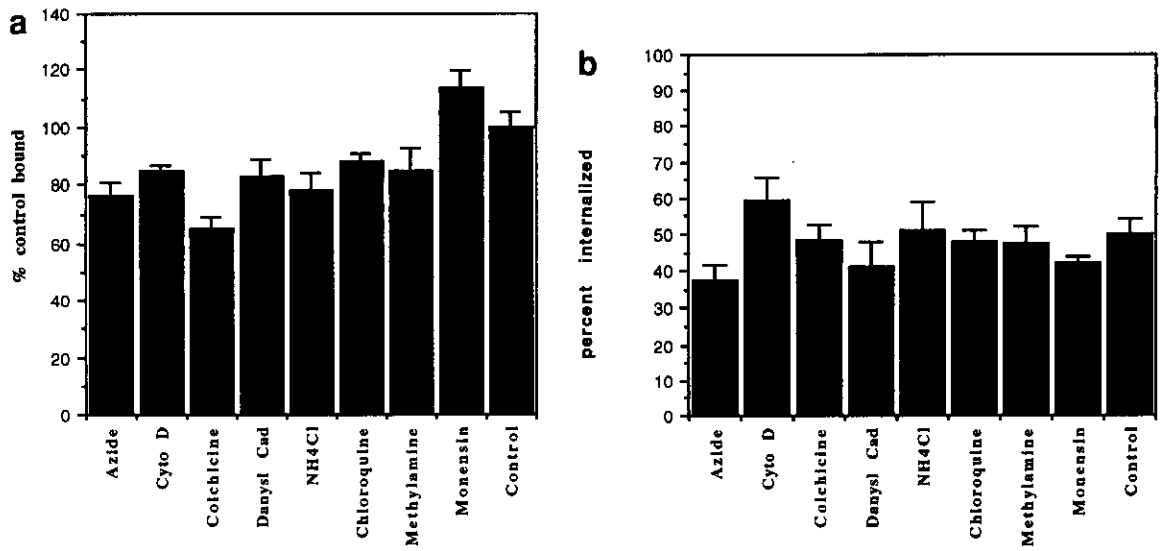

FIG. 6. (a) Effects of various drugs on L cell binding of radiolabeled RRV rotavirus. Binding was at 4° for 1 hr. Data are expressed as percentages of control (untreated) binding and represent means of three replicate monolayers with SEM indicated by the error bars. The concentration of the drugs used were: Na Azide, 100 m*M*; cytochalasin D, 20 μg/ml; colchicine, 1 m*M*; dansylcadaverine, 1 m*M*; NH_4Cl, 100 m*M*; methylamine, 50 m*M*; chloroquine, 10 m*M*. These concentrations were the highest tolerated by the cells in the previously described experiments. (b) Effects of various drugs on L cell internalization of radiolabeled RRV rotavirus. Binding was at 4° for 1 hr. Internalization was measured after a further 60 min at 37° with external virus removed by proteinase K digestion as described under Materials and Methods. Data are expressed as percentages of bound virus which were internalized and represent means of three replicate monolayers with SEM indicated by the error bars.

RRV to study the binding and internalization of rotavirus with L cell monolayers in the presence or absence of various drugs. In Fig. 6 it can be seen that none of the drugs had a significant effect on RRV binding to L cells at 4°. Similarly, Fig. 6 shows that RRV internalization by L cells as measured by escape from cell surface treatment with proteinase K was comparable for all of the conditions studied.

Electron microscopy suggests that dansylcadaverine inhibits endocytosis of rotavirus via coated pits

Thirty minutes after warming in untreated L cells, rotavirus particles were observed in coated pits and vesicles near the surface of the plasma membrane (Fig. 7). Rotavirus particles observed to be associated with the dansylcadaverine-treated cells were on the surface of the plasma membrane or rarely within vesicles without morphologic evidence of a clatherin coat. We did not observe definite morphologic evidence of direct membrane penetration.

Dansylcadaverine fails to enhance RRV infection of MA 104 cells regardless of trypsin activation state of virus

Because previous studies have suggested that proteolytic activation of rotavirus by trypsin facilitates membrane penetration of susceptible cells (Fukuhara *et al.*, 1987; Kaljot *et al.*, 1988), we wished to determine whether dansylcadaverine could enhance rotavirus, which had not been proteolytically activated in its ability to infect permissive MA 104 cells. The results, shown in Fig. 8, indicated no definate enhancement of either trypsin-activated or nonactivated virus by dansylcadaverine. As expected, trypsin activation increased infectivity approximately 10-fold.

DISCUSSION

The rotavirus replication cycle begins with cell attachment, followed by transport of the virus to the site of transcription and translation, the cell cytoplasm. It is believed that the low-calcium environment of the cell cytoplasm triggers uncoating of the virus to a transcriptionally active form, the single-shelled particle (Cohen *et al.*, 1979; Ludert *et al.*, 1987). The precise mechanism by which rotavirus reaches the cytoplasmic compartment is incompletely understood.

Previous ultrastructural studies have offered varying observations on the mode of rotavirus entry into cells. In several studies rotavirus particles have been observed in coated pits and vesicles in permissive MA 104 cells (Ludert *et al.*, 1987; Quan and Doane, 1983). In two other studies, trypsin-activated human rotavirus was reported to enter cells by direct penetration of the plasma membrane, while virus which had not been trypsin-activated entered via endocytosis (Suzuki *et al.*, 1985, 1986). All such ultrastructural studies must be interpreted with caution because particle to plaque-forming unit ratios for rotavirus preparations are typically greater than 100:1. Thus the great majority of observed virions will not be able to initiate infection. Several studies have docu-

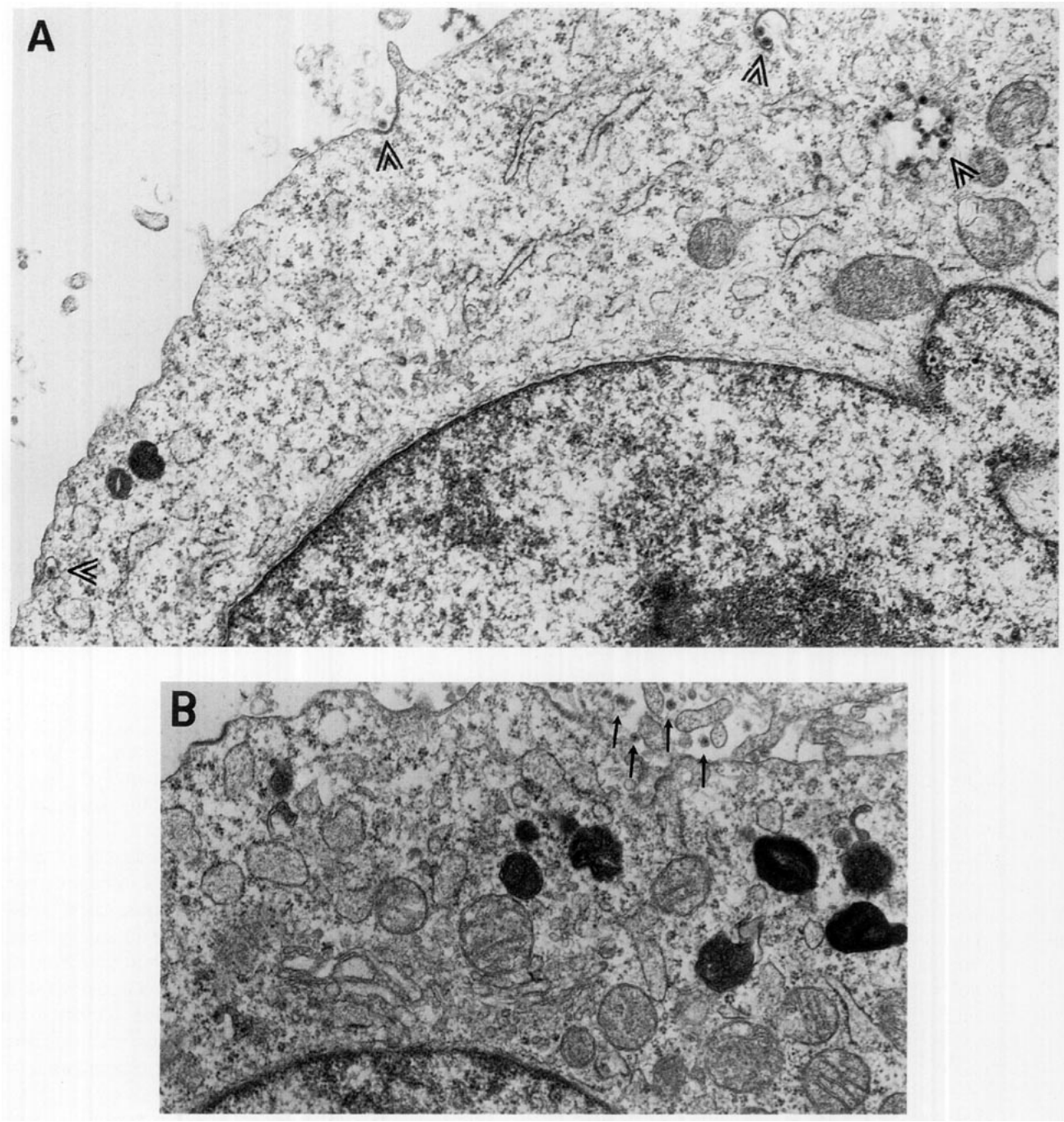

FIG. 7. (A) Electronmicrographs of control L cell 30 min postinfection with RRV rotavirus. Arrowheads indicate virions in coated pits and vesicles. Magnification 20,000×. (B) Electronmicrograph of an L cell, pretreated with 0.25 m*M* dansylcadaverine, 30 min postinfection with RRV rotavirus. Arrows indicate virions adherent to microvillus-like processes of the L cells. Magnification 20,000×.

mented the failure of lysosomotrophic agents to significantly inhibit rotavirus replication in permissive MA 104 cells (Fukuhara *et al.*, 1987; Kaljot *et al.*, 1988; Ludert *et al.*, 1987). Kaljot *et al.* (1988) reported that trypsin-treated rotavirus mediated release of chromium from preloaded permissive MA 104 cells, an observation which supports the direct membrane penetration hypothesis. Furthermore, chromium was not released from nonpermissive bovine aortic endothelial cells after rotavirus exposure. Fukuhara *et al.* (1988) reported biochemical evidence that trypsin-treated human rotavirus entered MA 104 cells by direct membrane penetration, while virus which was not trypsin-treated entered the cells by endocytosis and failed to uncoat.

More recently, we have examined a panel of cell lines including nonpermissive L cells as well as permissive MA 104 cells (Bass *et al.*, 1992). All of the cells bound and internalized radiolabeled rotavirus equally well as

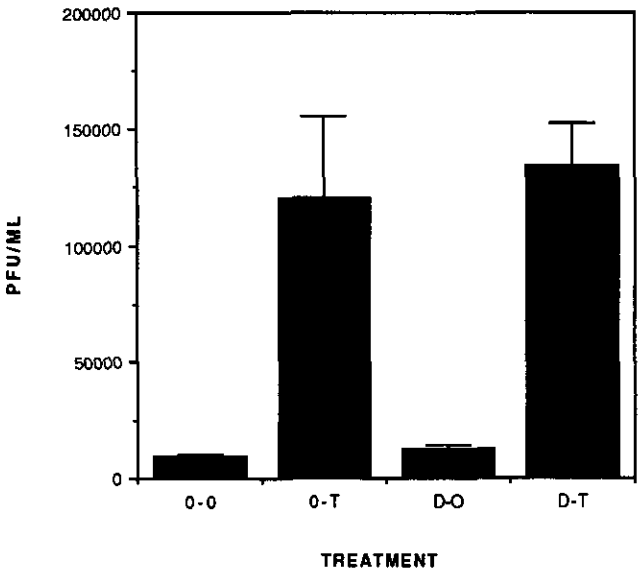

FIG. 8. Effects of dansylcadaverine 0.25 m*M* on infection of MA 104 cells by RRV rotavirus. Cells were pretreated with dansylcadaverine (D) or mock treated (O) for 1 hr prior to infection with either trypsin-treated (T) or mock-treated (O) RRV rotavirus.

measured by resistance to vigorous surface protease treatment. In nonpermissive L cells, a significant portion of the internalized virus remained in a fully infectious form for several hours after internalization. This was in contrast to the input virus in permissive MA 104 cells, in which an eclipse phase consistent with viral uncoating and transcriptional activation was noted. Finally it was shown that all of the cells were equally permissive for rotavirus if the virus was introduced via liposomes. These studies suggested that penetration of the plasma membrane determined the permissivity of the various cell lines and that in nonpermissive cells rotavirus was sequestered outside the cytoplasmic site of uncoating. The studies did not address whether membrane penetration into the cytosol occurred at the cell surface or from within endocytic vesicles.

The present experiments were initiated to attempt to further define the resistance of L cells to rotavirus infection and determine whether endocytosis is a productive route of infection. Because rotavirus appeared to be sequestered (possibly in the endocytic vesicles) in nonpermissive cells, we used a variety of drugs known to affect the endocytic process. Some of the drugs, such as ammonium chloride, methylamine, chloroquine, and monensin, act primarily to inhibit acidification of the endosome. Acidification of the endosome is essential for a variety of viruses, including reoviruses, to traverse the vesicle membrane into the cell cytoplasm (Sturzenbecker *et al.*, 1987). Although all of these drugs inhibited reovirus replication in L cells (Fig. 2), none of the drugs facilitated rotavirus replication (Fig. 1). Thus it seems unlikely that the lower pH of endosomes is the primary block to rotavirus penetration into the cytoplasm.

Colchicine has its primary effect on microtubules by inhibiting tubulin polymerization. Although microtubules are not generally thought to play an important role in endocytosis, others have shown that disruption of microtubule function can interfere with early phases of herpesvirus and simian virus 40 replication (Shimura *et al.*, 1987; Wittels and Spear, 1991). Direct observation of fluorescein-labeled reovirus containing vesicles in living cells has shown that colchicine inhibits their movement through the cytoplasm (Georgi *et al.*, 1990). Colchicine had no effect on replication of either rotavirus or reovirus (Figs. 1 and 2).

Dansylcadaverine may exert its effects on endocytosis by several mechanisms. As a weak base, it is able to prevent acidification of endosomes. It is also a very potent inhibitor of the transglutamase which cross-links clatherin subunits (Schlegel *et al.*, 1982). Thus, it has been reported to prevent clustering and internalization of receptors associated with coated pits (Haigler *et al.*, 1980; Schlegel *et al.*, 1982). Dansylcadaverine has also been reported to inhibit endocytosis of transferrin by preventing recycling of internalized receptors to the cell surface (Grasso *et al.*, 1990). In our experiments, dansylcadaverine was a potent inhibitor of reovirus infection (Fig. 2) and an enhancer of rotavirus infection in L cells (Fig. 1). While the inhibition of reovirus could be due to the drug's effect on endosomal pH, the fact that other agents which increase endosomal pH had no effect on rotavirus infection makes this mechanism unlikely for the enhancement of rotavirus infection. It seems much more likely that the drug is acting at an early stage of the endocytic process.

Cytochalasin D, like other cytochalasins, acts primarily by inhibition of actin polymerization. The role of actin microfilaments in endocytosis or phagocytosis of a number of invasive bacteria in cultured cells is well documented (Detilleux *et al.*, 1991; Elsinghorst *et al.*, 1989; Ewanowich *et al.*, 1989; Kuhn *et al.*, 1988). Cytochalasin also inhibits endocytosis of some toxins (Gordon *et al.*, 1988), interleukin 4 (Galizzi *et al.*, 1989), influenza virus (Gottlieb *et al.*, 1993), and herpesvirus (Rosenthal *et al.*, 1988; Wittels and Spear, 1991). Cytochalasins have also been shown to inhibit endocytosis in endothelial cells (Holland *et al.*, 1992) and from the apical but not the basolateral domain of polarized epithelial cells (Gottlieb *et al.*, 1993). In our experiments (Figs. 1 and 2), cytochalasin treatment of L cells enhanced RRV infection but inhibited reovirus infection.

Although both dansylcadaverine and cytochalasin have been reported to inhibit endocytosis in a variety of systems, neither drug is a specific inhibitor. It is possible that the enhancement of L cell rotavirus infection is due to some other effect of the drugs on the very early steps in rotavirus replication. Neither drug has a significant effect on total virus binding or internalization as grossly measured by resistance to treatment of the cell surface

with protease (Fig. 4). The drugs appear to exert their effect by altering the route of viral internalization. Time course studies confirmed that the drugs exert their influence in the very early stages of rotavirus infection (Fig. 5). Furthermore, dansylcadaverine decreased the proportion of internalized RRV which remained infectious (Fig. 6). This suggests that less virus was sequestered intracellularly in a site where uncoating does not occur, such as within endocytic vesicles. Since total virus internalized was equivalent, it stands to reason that a higher proportion of the virus in treated cells was not sequestered. This correlates with the increased efficiency of infection observed in the cells treated with dansylcadaverine and cytochalasin. Electron microscopy supports this hypothesis in that coated pits and vesicles containing virions were observed only in untreated L cells (Fig. 7), while dansylcadaverine-treated cells had only rare vesicles containing virions. The vesicles which were observed in the treated cells did not appear to be clatherin coated. The observations that both dansylcadaverine and cytochalasin enhanced rotavirus infection of L cells suggest that once rotavirus is internalized in endocytic vesicles, it can no longer efficiently infect the cells.

Of interest is the observation that the drugs had little effect on rotavirus infection of permissive MA 104 cells, whether the rotavirus had been trypsin activated or not. Endocytosis of rotavirus by MA 104 cells has been previously observed by electron microscopy (Ludert *et al., 1987; Quan and Doane, 1983; Schulze and Schumacher,* 1986). Others have observed the lack of effects of lysosomotrophic drugs on rotavirus replication in susceptible MA 104 cells (Fukuhara *et al.,* 1987; Kaljot *et al.,* 1988; Ludert *et al.,* 1987). Data from the currant and previous studies (Bass *et al.,* 1992) suggest that fewer infectious rotavirus particles are sequestered within MA 104 cells. It is possible that the L cell plasma membrane contains "pseudoreceptors" which direct rotavirus particles into a nonproductive endocytic pathway. It is also possible that rotavirus can infect MA 104 cells but not L cells via either direct penetration or pH-independent endocytosis. Whatever the mechanism, our results suggest major differences between early interaction between the plasma membrane and rotavirus between the two cell lines.

Finally, it should be noted that while endocytosis is a productive route of infection for many viruses in many types of cells, endocytosis can be a nonproductive route of entry for some viruses such as rotavirus or certain strains of mouse hepatitis virus (Kooi *et al.,* 1991). Thus endocytosis may function as a cellular defense mechanism against some viral infections.

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